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Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide

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Headspace-gas chromatography was employed to study the behavior of methanethiol in buffer solutions (pH 6·3) in the presence of transition metals and ascorbate. In the presence of Cu(II) (1 ppm), methanethiol (2 ppm) was depleted within 30 min at 30°C by 70% and 30% under aerobic and anaerobic conditions, respectively. Under similar conditions, Fe(III) alone catalyzed the oxidation of methanethiol to only a very limited extent ({ 15%). However, ascorbate plus Fe(III) mediated a rapid formation of dimethyl trisulfide at 30°C in a model system containing methanethiol and hydrogen sulfide. A partial inhibition of dimethyl trisulfide formation by benzoate in the ascorbate plus Fe(III) system suggested that the hydroxyl radical was involved in the pathway leading to the formation of dimethyl trisulfide.

INTRODUCTION

Cabbage, broccoli, sauerkraut, and other cruciferous vegetable foods often develop unpleasant off-flavors that shorten shelf life and lessen consumer acceptance. Volatile sulfur compounds are largely responsible for the off-flavors in these cruciferous vegetables (Maruyama, 1970; Forney *et al.*, 1991; Chin & Lindsay, 1993*a*). Methanethiol (CH_3SH) is an important contributor to these flavors because of its very low flavor-threshold value (0·02 ppb in water; Hansen *et al.*, 1992) and objectionable odor (Windholz *et al.*, 1983; Lindsay & Rippe, 1986), but it is also readily converted to even more unpleasant oxidized sulfurous off-flavor compounds (Miller *et al.*, 1973; Lindsay *et al.*, 1986; Hansen *et al.*, 1992).

It was observed earlier that ascorbic acid concentrations in cabbage were negatively correlated (−0·617) with methanethiol concentrations after tissue disruption (Chin & Lindsay, 1993*b*). Since ascorbate in the presence of transition metal ions may act as a pro-oxidant in lipid and protein systems (Kanner & Mendel, 1977; Mahoney & Graf, 1986; Uchida *et al.*, 1992; Uchida & Kawakishi, 1992), it was speculated that lower amounts of methanethiol in cabbage tissues may have resulted from an ascorbate- and metal-ion-mediated oxidation of methanethiol. Since ascorbate has been included in some strategies for the control of off-flavors in cruciferous vegetables, additional information is needed about the effects of ascorbate upon the volatile sulfur compounds. the objective of this study was therefore to determine the influence of transition metal ions and ascorbate on the oxidation of methanethiol and the development of oxidized methanethiol-related flavor compounds.

MATERIALS AND METHODS

Materials

Methanethiol and dimethyl trisulfide (CH_3SSSCH_3) were obtained from Eastman Fine Chemicals (Rochester, NY). Dimethyl disulfide (CH_3SSCH_3), sodium sulfide, ascorbic acid, and cupric chloride ($CuCl_2$) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Ferric chloride ($FeCl_3$) was obtained from Sigma Chemical Co. (St. Louis, MO). Chelex 100 ion exchange resin (100–200 mesh) was obtained from Bio-Rad Laboratories (Richmond, CA).

Serum-type vials (total volume 120 ml; Supelco Co., Bellefonte, PA) fitted with Mininert valve closures (20 mm diameter; Supelco) were used as reaction vessels. Methanethiol stock solutions were prepared in nitrogen-saturated methanol in serum-type vials sealed with Mininert valves. Hydrogen sulfide (H_2S) was prepared in a sealed vial by acidification of sodium sulfide (Whitten *et al.*, 1988). Deionized water from a Milli-Q Plus water-purification system (Millipore Co., Bedford, MA) was used to prepare all aqueous solutions.

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Methods

For determination of the effects of transition metal ions on the oxidation of methanethiol, 5 ml of sodium phosphate buffer solution (50 mM, pH 6·3) which had been previously demetallized by passage through a column of Chelex 100 ion-exchange resin were added to each reaction vial. Vials were then purged (30 ml/min for 15 min) with either purified air or nitrogen to achieve aerobic or anaerobic conditions, respectively, and sealed under continuing gas flow with Mininert valves. After sealing, vials were held at 30°C in a water bath (Cambridge Instruments, Buffalo, NY). Metal ions (58 μg $FeCl_3$, equivalent to 4 ppm Fe(III) for the solution; or 10·6 μg $CuCl_2$, equivalent to 1 ppm Cu(II)) from freshly prepared stock solutions were added to each of the temperature-equilibrated vials just prior to the addition of methanethiol. Reactions were initiated by adding methanethiol (10 μg, equivalent to 2 ppm) to each vial from the chilled (0°C) methanolic stock solution with a 10–μl gas-tight syringe (Hamilton Co., Reno, NV).

The disappearance of methanethiol from reaction vessels was monitored by headspace gas chromatography. Headspace gas (0·3 ml) from each reaction vial was periodically injected with a 1–ml gas-tight syringe (Hamilton) into a Varian (Palo Alto, CA) 3700 gas chromatograph (GC) equipped with a flame photometric detector (FPD) and a glass column (6 ft × 2 mm i.d.) packed with 40/60 Carbopack B HT 100 (Supelco). Production of dimethyl disulfide and dimethyl trisulfide was analyzed by injecting a larger amount (2 ml) of the headspace gas from each reaction vial after holding 300 min at 30°C. An isothermal oven temperature of 40°C was used for elution of methanethiol. For elution of dimethyl disulfide and dimethyl trisulfide, the oven temperature was held initially at 40°C for 1 min, programmed to 180°C at 20°C/min, and finally held at 180°C for 5 min. Both the injector port and detector were maintained at 200°C. Helium at 30 ml/min was used as the carrier gas. A Varian 4270 integrator was employed to acquire data.

The effects of ascorbate plus metal ions on the oxidation of methanethiol and the development of oxidized compounds were studied by preparing a series of reaction vials containing 50 ml phosphate buffer solution (50 mM, pH 6·3). Vials were purged with purified air (30 ml/min) for 20 min before sealing with Mininert valves. Ascorbate (450 ppm), Fe(III) (4 ppm), and hydrogen sulfide (1 ppm) were added to each vial equilibrated at 30°C, and then methanethiol (1 ppm) was added to initiate reactions. At various times after the addition of methanethiol, 4 ml headspace gas samples from reaction vials were analyzed by GC for dimethyl disulfide and dimethyl trisulfide.

Quantifications of methanethiol, dimethyl disulfide, and dimethyl trisulfide were accomplished by preparing standard curves with authentic compounds in phosphate buffer solutions (50 mM, pH 6·3). A plot of the square root of peak area versus the total mass (μg) of the sulfur compound gave a linear relation. Samples were analyzed in duplicate, and the coefficient of variation was less than 5% for each mean value.

RESULTS AND DISCUSSION

Effects of Cu(II) and Fe(III)

The presence of Cu(II) (1 ppm) caused a rapid disappearance of methanethiol (2 ppm) from both aerobic and anaerobic samples (Fig. 1), whereas only a slight loss of methanethiol occurred in samples containing Fe(III) (4 ppm). The concentration of copper in cabbage reported by Pennington (1976) was about 1 ppm, and Cu(II) at this level in the model systems reduced methanethiol concentrations by 70% and 30% within 30 min at 30°C under aerobic and anaerobic conditions, respectively (Fig. 1). Pennington (1976) reported that iron was present in cabbage at about 4 ppm, but Fe(III) at this level resulted in the removal of less than 15% of methanethiol (2 ppm) over a period of 300 min in either aerobic or anaerobic samples (Fig. 1). In the control samples, the rates of disappearance of methanethiol were low, but the rate of disappearance was somewhat greater in the presence of air than in the nitrogen-saturated sample.

Only dimethyl disulfide was detected as a volatile product of methanethiol oxidation catalyzed by metal ions, and dimethyl trisulfide was not detected throughout the experimental period (300 min). This is in agreement with the finding (Chin & Lindsay, 1993*b*) that hydrogen sulfide was needed to provide a sulfur element in the oxidative formation of dimethyl tri-sulfide from methanethiol.

Dimethyl disulfide accounted for only part of the methanethiol that had disappeared from the samples (Table 1). In the aerobic sample containing Cu(II), only 51% of the methanethiol that disappeared from the headspace was converted to dimethyl disulfide (Table 1). The remaining portions of the methanethiol that disappeared from the headspace gases were unaccounted

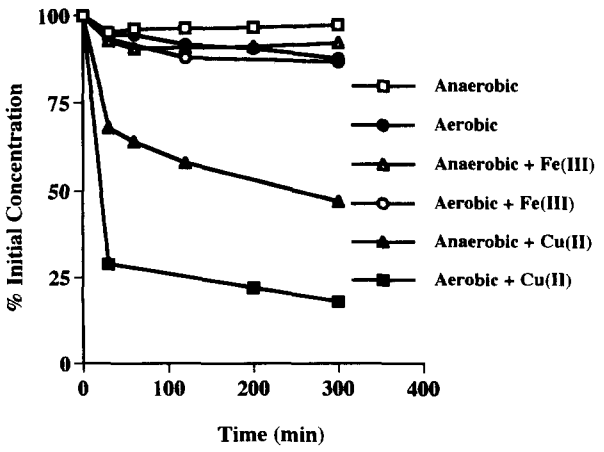

Fig. 1. Rates of disappearance of methanethiol (2ppm) catalyzed by Fe(III) (4ppm) and Cu(II) (1 ppm) under aerobic and anaerobic conditions at 30°C.

Table 1. Fe(III) and Cu(II)-catalyzed conversion of methanethiol (2 ppm) to dimethyl disulfide after 300 min at 30°C

Sample	Amount (ppm)		% Conversion of CH_3SH to CH_3SSCH_3 (Production/loss × 100)
	Loss of CH_3SH	Production of CH_3SSCH_3	
Fe(III) aerobic	0·22	0·17	77
Cu(II) aerobic	1·64	0·84	51
Cu(II) anaerobic	1·06	0·46	43
Cu(II) ascorbate anaerobic	0·87	0·20	23

for, but they could have been converted to oxygen-bearing compounds that were not sufficiently volatile to be detected by the headspace-GC method. Methanesulfenic acid (CH_3SOH), methanesulfinic acid (CH_3SO_2H), and methanesulfonic acid (CH_3SO_3H) are included in this group of compounds. The formation of oxygen-bearing products from air oxidation of thiols in the presence of metal ions or oxidation by strong oxidants (e.g. H_2O_2) is well documented (Little & O'Brien, 1967; Harman *et al.*, 1984; Mason & Rao, 1990).

However, thiols have also been shown to react with Cu(I) to form non-volatile cuprous mercaptides (R-S-Cu; Swan, 1965). Hanna and Mason (1992) have shown that Cu(I) is stabilized by reduced glutathione through a chelation mechanism. Thus, if Cu(I) was formed from the oxidation of methanethiol by Cu(II) through direct electron transfer (Harman *et al.*, 1984; Rhee *et al*, 1990), it could have been responsible for the unaccounted methanethiol. The fact that the methanethiol that disappeared from the sample containing Cu(II) under anaerobic conditions was only partially converted to dimethyl disulfide (Table 1) suggested that Cu(I) reacted with methanethiol to form mercaptides.

Ascorbic acid has been shown to reduce Cu(II) to Cu(I) (Swan, 1965; Gardner & Lawrence, 1993). When ascorbate (10 ppm) was included in the anaerobic samples containing methanethiol (2 ppm) and Cu(II) (1 ppm), fewer portions (23%, Table 1) of the methanethiol that had disappeared from the headspace were accounted for by dimethyl disulfide as compared with the samples without ascorbate (43%, Table 1). This further supported the belief that Cu(I) formed mercaptides with methanethiol, resulting in a reduced production of dimethyl disulfide.

Iron did not appear to react similarly to copper because aerobic samples of methanethiol with or without Fe(III) produced equivalent amounts of dimethyl disulfide after complete disappearance of methanethiol (data not shown). In the absence of ascorbate, copper, but not iron, would therefore be expected to influence the development of sulfurous off-flavors in foods. Cu(I) could suppress methanethiol malodors by forming the non-volatile mercaptide, but Cu(II) could catalyze the oxidation of methanethiol to dimethyl disulfide, which also possesses an undesirable putrid note (Lindsay *et al.*, 1986).

Effects of Ascorbate plus Fe(III)

The effects of ascorbate on the oxidation of methanethiol and the development of oxidized compounds were examined by including ascorbate (450 ppm) and hydrogen sulfide (1 ppm) in aerobic samples containing methanethiol (1 ppm) and Fe(III) (4 ppm). Ascorbate has been reported to be present in cabbage at about 450 ppm (Pennington, 1976). Hydrogen sulfide is present in disrupted tissues of cabbage at about 0.5 ppm (Bailey *et al.*, 1961; Chin & Lindsay, 1993*a*), and it is involved in the formation of dimethyl trisulfide by reacting with methanesulfenic acid (Maruyama, 1970; Chin & Lindsay, 1993*b*). Chin & Lindsay (1993*b*) have also shown that hydrogen sulfide reacts with the anhydride of methanesulfenic acid, i.e. methyl methanethiosulfinate ($CH_3S(O)SCH_3$), to yield dimethyl trisulfide. Hence, hydrogen sulfide was included in the model system at a level approximating to that in disrupted cabbage tissues to test whether ascorbate could facilitate the oxidative formation of dimethyl trisulfide from methanethiol.

The presence of ascorbate plus Fe(III) resulted in a rapid formation of dimethyl trisulfide from methanethiol (Fig. 2). Cu(II) had similar effects on the oxidative formation of dimethyl trisulfide from methanethiol (data not shown). These results are compatible with the observation that initial concentrations of ascorbic acid in macerated cabbage were negatively correlated with methanethiol production in a variety of cultivars (Chin & Lindsay, 1993*b*). The concentration of dimethyl trisulfide produced in samples containing ascorbate plus Fe(III) was about seven times that in samples with only Fe(III) after 330 min at 30°C (Fig. 2). In the

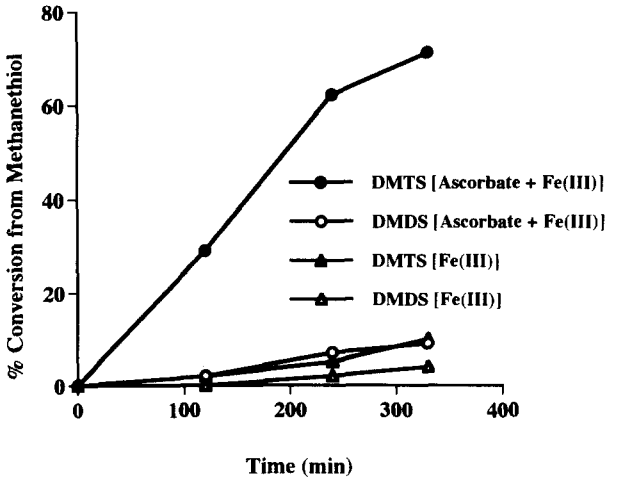

Fig. 2. Effects of ascorbate (450 ppm) plus fe(III) (4 ppm) and Fe(III) alone (4 ppm) on the rats of formation of dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) from methanethiol (1ppm) and hydrogen sulphide (1ppm) under aerobic conditions at 30°C.

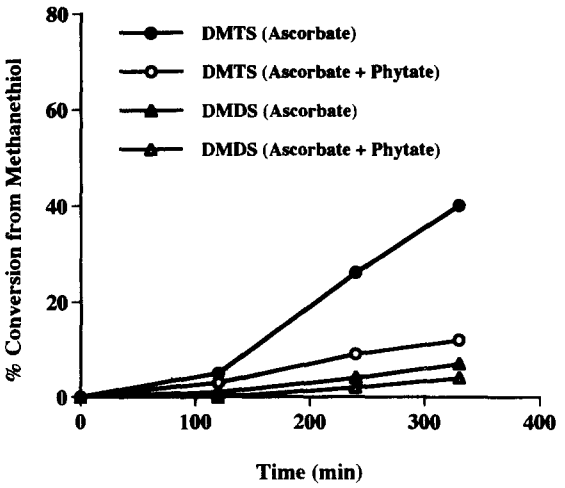

Fig. 3. Effects of ascorbate (450 ppm) plus phytate (1 mM) and ascorbate alone (450 ppm) on the rates of formation of dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) from methanethiol (1 ppm) and hydrogen sulphide (1 ppm) under aerobic conditions at 30°C.

presence of ascorbate alone, production of dimethyl trisulfide (Fig. 3) was reduced by 45% after 330 min (30°C) compared to samples containing both ascorbate and Fe(III) (Fig. 2).

Although stock solutions of ascorbate had been demetallized with a column of Chelex 100 ion exchange resin, minor post-column contamination of samples by metals might still have occurred. Furthermore, the efficacy of Chelex 100 has been questioned in relation to its use in oxygen radical research (Schaich, 1990). When phytic acid (1 mM, Aldrich Chemical Co.), a chelating agent that forms catalytically inactive iron chelates (Mahoney & Graf, 1986), was included in samples containing ascorbate, the production of dimethyl trisulfide was reduced by 75% (330 min at 30°C) as compared with samples containing ascorbate alone (Fig. 3). Thus these results indicated that catalytically active metal ions are required for the ascorbate-mediated oxidative formation of dimethyl trisulfide from methanethiol. In contrast, dimethyl disulfide was produced in very low and similar concentrations in all samples (Figs 2 and 3).

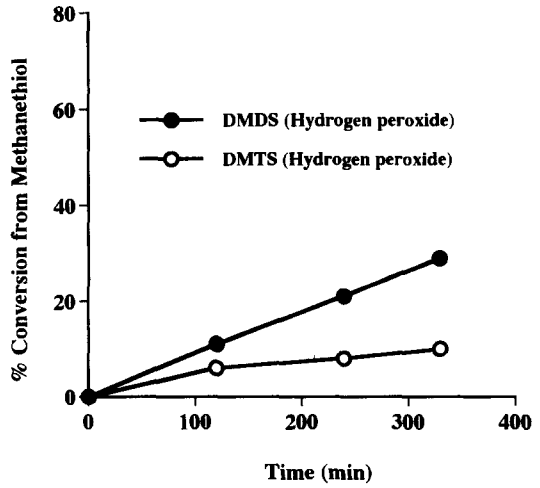

Fig. 4. Effect of hydrogen peroxide (100 oom) on the rates of formation of dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) from methanethiol (1 ppm) and hydrogen sulfide (1 ppm) under aerobic conditions at 30°C.

Methanethiol and hydrogen sulfide in cruciferous vegetables (Bailey *et al.*, 1961; Forney *et al.*, 1991; Chin & Lindsay. 1993*a*) and other foods (Shankaranarayana *et al.*, 1982) are derived from sulfur-containing amino acids either by microbial or enzymic actions (Herbert *et al.*, 1971; Miller *et al.*, 1973) or by chemical reactions (Schutte, 1974; Shankaranarayana *et al.*, 1982). The results of the current studies indicated that the concentration of ascorbate in foods is likely to be influential in the development of oxidized sulfurous off-flavors caused by dimethyl disulfide and dimethyl trisulfide, which have been shown to be responsible for putrid-type aromas in spoiled fish (Lindsay *et al.*, 1986) and spoiled ground beef (Stutz *et al.*, 1991).

In another experiment, methanethiol (1 ppm) in the presence of hydrogen sulfide (1 ppm) and hydrogen peroxide (H_2O_2; 100 ppm) also yielded dimethyl trisulfide (Fig. 4). However, the relative amounts of dimethyl disulfide and dimethyl trisulfide were different in the presence of H_2O_2 (Fig. 4) from what they were in the presence of ascorbate plus Fe(III) (Fig. 2). The concentration of dimethyl disulfide was higher than that of dimethyl trisulfide in the H_2O_2 system (Fig. 4), whereas dimethyl trisulfide was the predominant product when ascorbate plus Fe(III) was present (Fig. 2).

The role of H_2O_2 in the oxidative conversion of methanethiol to dimethyl disulfide and dimethyl trisulfide was further investigated because it could also have been involved in the ascorbate plus Fe(III) system. Aerobic oxidation of ascorbate in the presence of transition metals has been shown to produce H_2O_2 (Bauernfeind & Pinkert, 1970), which could give rise to the hydroxyl radical ($^{\cdot}OH$) via the Fenton reaction (Korycka-Dahl & Richardson, 1978). When benzoate (10 mM), a hydroxyl-radical scavenger (Korycka-Dahl & Richardson, 1978), was included in the ascorbate plus Fe(III) samples, the production of dimethyl disulfide and dimethyl trisulfide was inhibited by 42% and 50%, respectively, after 330 min at 30°C (data not shown). These results suggested that the hydroxyl radical was involved, and perhaps was the predominant active-oxygen species, when ascorbate plus Fe(III) mediated the oxidative formation of dimethyl trisulfide from methanethiol. A proposed mechanism for the formation of dimethyl trisulfide involving ascorbate and Fe(III) is shown in Fig. 5.

In the proposed mechanism (Fig. 5), a transition-metal ion such as Fe(III) could initiate methanethiol oxidation by accepting an electron from the thiol to form Fe(II) and a thiyl free radical (Reaction *a*). Ascorbate could also reduce Fe(III) to Fe(II) as shown in Reaction *b*. The production of hydrogen peroxide (Reaction *c*) from the aerobic oxidation of ascorbate in the presence of transition-metal ions has been documented by Bauernfeind and Pinkert (1970). Fe(II) may react with hydrogen peroxide to yield the hydroxyl

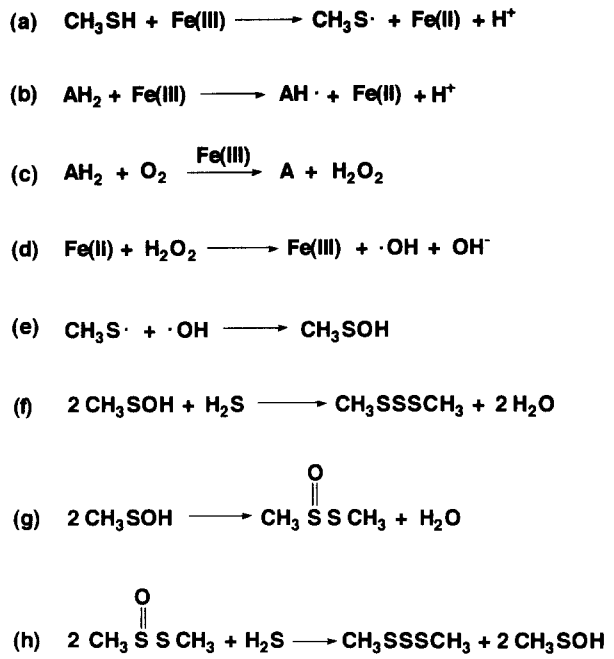

Fig. 5. Proposed mechanisms for the ascorbate (AH_2)-plus-Fe(III)-mediated formation of dimethyl trisulphide from methanethiol and hydrogen sulphide under aerobic conditions; A = dehydroascorbate.

radical (Reaction *d*) according to the Fenton reaction (Korycka-Dahl & Richardson, 1978). Hydroxyl radicals are very reactive and could react rapidly with thiyl free radicals to form a sulfenic acid (Reaction *e*). Dimethyl trisulfide would then arise from a reaction of the sulfenic acid with hydrogen sulfide (Reaction *f*) as proposed by Maruyama (1970). Alternatively, two molecules of sulfenic acid may dehydrate to form methyl methanethiosulfinate (Reaction *g*; Penn *et al.*, 1978). Methyl methanethiosulfinate has been shown to react rapidly with hydrogen sulfide to yield dimethyl trisulfide (Reaction *h*; Chin and Lindsay, 1993*b*).

Although ascorbic acid is employed as an antioxidant in food processing (Bauernfeind & Pinkert, 1970; Lewis, 1989), its use in some foods has been questioned (Kanner & Mendel, 1977; Mahoney & Graf, 1986; Uchida *et al.*, 1992; Uchida & Kawakishi, 1992). In the current model-system studies, ascorbate did not exert antioxidant activity against the oxidation of methanethiol under aerobic conditions. Instead, ascorbate served as a pro-oxidant that resulted in the rapid formation of dimethyl disulfide and dimethyl trisulfide.

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